The end or the beginning of the drive to an HIV-preventive vaccine: a view from over 20 years

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Richard Horton has seriously questioned the possibility of developing a successful HIV-preventive vaccine and has appropriately criticised an over optimistic attitude promoted by some individuals in the HIV-vaccine research community.[1] Horton's piece is, for the most part, on the mark and useful. However, there are points that I believe will benefit from clarification. Whereas he rightly points out that some people working on HIV made glib statements for an early vaccine success, this position was never that of most leading HIV scientists. Indeed, many AIDS scientists regularly acknowledge the possibility of the impossibility of success. Horton implies that the International AIDS Vaccine Initiative (IAVI) once held an optimistic position, but most scientists recognised that this was naive, and IAVI, like most scientists, no longer argues that a vaccine is just around the corner. Correctly, Horton criticises a vaccine efficacy trial,[2] but it is noteworthy that most AIDS scientists were also not pleased that this trial went forward and were keenly aware that it had little or no chance of success. I think we can all agree that continued promotion, testing, and predictable failure of vaccines that are not evidence-based will continue to foster negative views about HIV-vaccine development, thus leading some to the conclusion that vaccine research should be curtailed. There is no doubt that successful development of an HIV-preventive vaccine does present some unusual obstacles.

First, there are major challenges surrounding vaccine design. An HIV vaccine cannot consist of attenuated, actively replicating (live) HIV, even though the best vaccines for other viral diseases have usually used live viruses. There is inherent danger that attenuated HIV would cause AIDS.[3,4] Killed whole virus, usually the next best approach,[5] might also be precluded both because of the hazard (one cannot be certain that all virus particles would be inactivated), and because killed whole HIV has worked poorly in animal tests. New approaches to develop killed HIV vaccines that could be more immunogenic are underway. However, at this stage, and probably permanently, we are restricted by the need to use one or more HIV proteins or peptides (subunit vaccines) or their DNA form with or without delivery by various vectors. Although medical science has been successful with subunit vaccines,[6] we remain far less experienced with those approaches.

A second obstacle to an HIV-preventive vaccine is that we have no truly useful small animal model for studying HIV infection. Consequently, our efforts are limited to a relative of HIV—namely, the monkey virus SIV—with the assumption that what is learned from one virus is applicable to the other. More importantly, this model needs infection of monkeys, which are both expensive and available to very few investigators.

A third apparent problem is that we do not know with certainty which immune response will provide protection. Although this is also true for some vaccines that have already proven successful, their success often came from trial and error. Because the empirical approach has not worked for HIV, much emphasis in AIDS-vaccine research has been placed on finding correlates of immune protection in the SIV monkey models and studying individuals who show natural resistance to HIV. The former approach has the limitations that once again it focuses on SIV in monkeys and not HIV in man, and both approaches have the limitation that correlates are just that: they do not prove a mechanism for protection, although they could be worth pursuing. Moreover, what we are learning about natural resistance to HIV infection is that the correlate can in many cases be associated with innate immune responses. Unlike adaptive immune responses, such as specific antibodies and cell-mediated immunity (CMI) to HIV, innate immunity lacks a typical memory response, so specific recall is difficult to induce. Consequently, we would have to sustain an innate immune response for years and even decades, but we do not know how to achieve this end or whether a heightened sustained response would be harmful.

A fourth difficulty is HIV strain variation. Although we have known about HIV variation among isolates since 1984,[7,8] and about important microvariants within an isolate from a single individual since 1988,[9] new recombinant forms continue to emerge.[10] Probably no single obstacle has raised more concern than this one, although for reasons discussed below there is room for hope.

The fifth, and in my view the most important obstacle, is that HIV as a retrovirus integrates its genes into the target cell DNA, thereby quickly establishing a lifelong infection if not stopped at the time of initial exposure. HIV is sometimes noted to be unique among virus infections in that there is no case of documented complete viral clearance. However, this is not so; rather, it is generally true for all retroviruses, including other human retroviruses such as HTLV-1. Consequently, ever since we knew that HIV was the cause of AIDS in 1984, this characteristic drove many researchers in the specialty to strive for sterilising immunity (complete protection against infection), or close to this goal, so that the cellular arm of the immune system has a fighting chance to remove a few infected cells. In my view, this

goal must be achieved to have a successful HIV-preventive vaccine. To reach this goal, most researchers in the early years focused on induction of antibodies to HIV gp120 envelope because some of those antibodies can block HIV entry into cells, thereby providing a theoretical basis for sterilising immunity. These antibodies are the neutralising antibodies. This notion was soon experimentally verified in the SIV monkey model, and until the late 1980s fostered the hope that sterilising immunity could be achieved by using gp120 envelope vaccines. The bubble burst when researchers realised that protection was only against the exact strain of HIV used to produce the gp120 vaccine or very closely related strains. One reason for this finding is the hypervariable region of gp120, called the V3 loop,[11] which HIV seems to use as an immunological decoy. The antibody response is strongly misdirected to this region and cannot control other HIV strains with different envelopes. Nonetheless, a phase III human efficacy trial was undertaken with such a vaccine candidate (gp120 from cell-line adapted HIV strains) and, predictably, it failed.[12]

A sixth problem, but actually a correlate of the problem resulting from HIV integrating its genes into the genes of the target cell, is that the immune response generated by the vaccine will probably have to be sustained. For vaccines against other viruses, the time it takes for memory cells to respond generally does not prevent ultimate clearance of the virus. However, HIV infection will probably be established by this time.

The seventh problem is that if sterilising immunity is not achieved, HIV infection begins to dampen the immune response against it soon after the establishment of infection. This occurrence is not restricted to adverse effects on HIV-infected cells because uninfected cells are also impaired. The effects might be mediated by the HIV Tat protein[13,14] released from acutely infected cells.[15,16]

As a result of the problems with gp120-based vaccine candidates, a consensus seemed to have been reached by the early 1990s, which in its simplest version states that, since sterilising immunity does not seem possible, CMI-based strategies should replace strategies based on gp120 neutralising antibodies. The logical corollary to this is that we must accept a vaccine that does not block infection. Rather, success is achieved by reducing the amount of HIV with the expectation that it will remain reduced and disease may well be prevented. Indeed, this view is common in the discipline today. Consequently, several CMI-based vaccine trials are now going forward and many use the same vector (modified vaccinia virus Ankara) to deliver various HIV genes. Although, this is where the specialty stands at present, and although these trials have some rational bases, the first candidate was not even significantly immunogenic. Also, although concentrations of SIV or SHIV have been reduced by such vaccines in monkeys, not surprisingly, viral escape mutants ultimately developed and disease occurred, albeit later than in unvaccinated groups.[17] It has been said that if similar results could be obtained in human beings, it would be a great advance[18] since such findings would show partial efficacy and a possible reduction in virus spread because the viral load in infected individuals would be low. However, in primates CMI vaccines do not reduce peak, acute viraemia by very much. CMI vaccines are chiefly promoted for reducing the chronic viral set point, and if the situation is the same in human beings as we suspect, this reduction might not be so helpful because transmissions could occur during peak acute viraemias. Moreover, these vaccines could lead to a false sense of security among vaccinees, and thus possibly to a greater number of infections. Nonetheless, there is a possibility that several similar trials will go forward. In fact, there is one in progress in Thailand, which has brought this healthy debate to a head.[19–23] The major issue is how to be sure that scientific rationale is the major motivation for clinical testing so that we can optimise the use of limited resources and preserve the goodwill from public and private funders of these programmes.

What then of the near future, and how can we overcome the scientific obstacles to an HIV vaccine outlined above and raised anew?[1] 1) Although we face restrictions on the forms of acceptable HIV vaccines, subunit vaccines are acceptable, and there are now documented success stories with subunit vaccines for other viruses.[6] 2) The SIV or SHIV monkey models, although limited to very few investigators, can be made much more available. This is a matter only of policy and funding for primate centres. There are assumptions needed for studies using SIV or SHIV instead of HIV, but these assumptions are probably valid because of the genomic and biological similarities of SIV and HIV. 3) Although we do not know the correlates of protection against HIV infection in most naturally exposed but uninfected human beings or in most studies of SIV infection of monkeys, a correlate does not constitute a proven mechanism. Also, generally we have little or no evidence of any correlate even for the most successful vaccines. Instead of focusing on finding the elusive correlate, obtaining or approaching sterilising immunity should be the goal; both conceptually and experimentally we know of only one practical way to accomplish this, namely—by eliciting neutralising antibodies that are broadly reactive against various HIV strains and that are expressed for long periods. In my judgment, instead of emphasising discovery of correlates of protection in SIV-monkey models, we need focused programmes to develop ways to elicit and maintain these antibodies. 4) Although the importance of the problem of the variability of HIV cannot be over emphasised, there is hope. We know of conserved regions of the HIV genome that are needed for successful HIV replication. We also know that to initiate infection, most HIV strains and

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their microvariants need gp120 binding to the coreceptor CCR5, after an initial interaction with CD4 as the target cells. Progress in the science of HIV entry has provided us with new opportunities to target gp120 sites or sites on a gp120–CD4 complex, which prevent gp120 interactions with CCR5. These sites are likely to be common among the various HIV strains, and this conclusion is lent support by tests of antibodies produced by gp120–CD4 complexes.[24–28] 5) As I have already emphasised, integration of the genes of a retrovirus into the cellular DNA of the target cell soon after infection may require a successful vaccine to block HIV infection from the onset and approach complete protection. Because there is little time to recall an immune response, the immunity must be sustained. The obvious way to do this is by a mechanism that blocks HIV entry into its target cell and does so for a long period.

We know of two ways to block HIV entry. One way is to reduce surface exposure of the CCR5 HIV coreceptor with its ligands, the β chemokines (RANTES, MIP-1 α, and MIP-1 β),[29] or other compounds.[30] We need to learn more about the regulation of chemokine genes and how to manipulate their expression. However, with the exception of the novel approaches described by Lehner and co-workers,[31] this path has not been taken up by HIV-vaccine workers. On the other hand, chronic stimulation of chemokine production might cause undesirable side-effects, as noted for general activation of innate immune responses, and how high-level β chemokine production could be sustained is unclear. As noted above, a second, more conventional approach is to induce broadly neutralising antibodies. Passive transfer of neutralising monoclonal antibodies has given complete protection of challenged monkeys. Thus, we know humoral immunity can provide sterilising immunity.[32] Also, recent studies have indicated that the HIV strains that first enter an individual, before the immune response, could be more vulnerable to antibody attack than the HIV strains we study that are isolated from patients after an established infection.[33] Although there have been major obstacles in inducing these antibodies, recent results provide reason for encouragement. Recent studies of HIV entry have yielded fundamental knowledge that affects rational vaccine design,[34,35] especially since these designs relate to vaccine approaches that are based on the development of neutralising antibodies. Partly on the basis of these results, animals were vaccinated with complexes of envelope gp120 and soluble human CD4,[24–28] which resulted in the production of antibodies that not only neutralise primary HIV isolates (those not adapted to cell lines but obtained from patients and grown only transiently in blood cells), but also HIV isolates from widely divergent clades. The concept is based on the stabilisation of a mobile envelope and fixation in a transition state that binds coreceptor. This transition state is an invariable antibody target since almost all HIV envelopes bind to CCR5 for HIV to enter cells and initiate infection. Antibodies raised against these complexes have been suggested to be directed to CD4[36] and would, therefore, be unattainable in human beings (because of tolerance) or unacceptable to the US Food and Drug Administration; neither premise is lent support by the evidence.[24–28] A report from Varadarajan and colleagues[37] revisited the issue by providing evidence that neutralising antibody responses raised against gp120–CD4 complex can be mediated by anti-CD4 antibodies. However, these findings are neither novel nor without serious limitations. In the first place, these workers used a human CD4 linked with gp120 injected into rodents (guinea pigs). Not surprisingly, in this xenogeneic heterologous system, high-titre anti-human-CD4 antibodies were generated, including ones that can broadly neutralise various strains of HIV, as has been described previously by Celada[24] and by DeVico[26] and their colleagues. Furthermore, these workers used DNA and DNA prime-boost vaccine approaches. The use of DNA to deliver a constrained structure is inherently problematic and may yield unpredictable products (unpublished). Our experience with these complexes is that when more homologous systems are used, the neutralising antibodies generated are mainly complex specific. Alternatively and preferably, the CD4 binding sequences could be replaced by a mimetic, but to date no mimetic has been obtained that produces the needed concentrations of antibodies. Thus, the study by Varadarajan and co-workers[37] only reconfirmed that human CD4 is highly immunogenic in rodents and should not obscure the potential of immune responses to constrained or transition state structures. The extent of the problem depends on the immunogen quality and nature of the test system. Unfortunately, Varadarajan and colleagues used one of the poorest systems, which was no more advanced than the original effort of Franco Celada and colleagues in 1990.[24] Better approaches include: 1) staying with CD4 as the structure-triggering agent but working in homologous systems,[38] including primate systems in which human CD4 (or primate CD4) is far less immunogenic than in other systems (unpublished); 2) replacing CD4 with a mimetic that triggers similar structural changes; or 3) use of other novel peptides and compounds to trigger the gp120 transition state. In the end, however, we acknowledge that achieving the needed levels of neutralising antibodies and being able to sustain them is not yet within our grasp.

Several other groups are pursuing advanced alternative approaches aimed at obtaining neutralising antibodies with the breadth needed for a successful vaccine, mainly through exploitation of epitope mapping with the aid of HIV neutralising monoclonal antibodies,[36] by deriving a consensus envelope sequence,[39] or by grouping of multiple envelopes (the cocktail approach).[40] I believe we are at the threshold of solving the problem of variation

and that sterilising immunity will be possible, though not yet in hand. Since administration of monoclonal antibodies that neutralise SHIV completely protects monkeys from infection, I believe we can and must assume that the same will be true of HIV. If by vaccination we generate such antibodies with sufficient titre, sustainability, and distribution to mucosal sites, then we should have a vaccine that achieves or approaches sterilising immunity.

Sustaining adequate levels of these broadly reactive antibodies or, for that matter, any HIV-envelope based antibodies has not been possible. As frequently noted by my colleague, George Lewis, solving this key scientific problem has, curiously, been neglected, and resolution probably relies on the use of a particular adjuvant or on its mode of delivery—a scientific problem ripe for experimentation.

Because some HIV variants might escape our best efforts at sterilising immunity, and a low level infection could occur, a cell-mediated immune response that generates killer T cells that can eliminate the few infected cells should be part of the vaccine strategy. Additionally, since many studies show that HIV infection can quickly dampen the immune response, and that this process could be mediated by the extracellular form of the HIV Tat protein, I suggest that vaccinating against the HIV Tat protein should also be part of our strategy.[13,14,41,42] Although several groups have included the *tat* gene in their DNA prime-boost strategy, this vaccine will chiefly generate a CMI response to Tat epitopes produced in infected cells. Although this approach could be helpful by providing additional CMI targets, it is hardly comparable to the CMI targets induced by the much more prevalent HIV structural proteins. When I suggest a vaccine that includes the targeting of the HIV Tat protein I do not refer to targeting Tat in HIV-infected cells. Rather, our focus on Tat is of the extracellular Tat, which is released from acutely infected CD4+ T cells, rapidly taken up by nearby cells, and contributes to a dampening of the immune response of these uninfected cells.[14] Therefore, in this context I call for including in our vaccines a component that induces high titre antibodies to Tat that interfere with its extracellular effects. This goal is best achieved with a Tat protein vaccine and not with a DNA construct that includes the *tat* gene.

In conclusion, it is not time to give up on HIV vaccines but to change the way we pursue them. HIV-preventive vaccine research should clearly ignore empirically driven approaches and rely exclusively on rational approaches that are based on solid knowledge of HIV biology. I suggest after years of wandering, we have only begun to travel this path. A shift in emphasis to solving key scientific problems that have plagued the field is necessary. How should such an expanded effort be realised and administered? In the USA, a few new large-scale funding approaches have recently come to the forefront as promising models. One, from the Gates Foundation, known as the Grand Challenges for Global Health, targets not only HIV, but also a select few microbes plaguing much of the developing world (eg, malaria and TB). In this scientist-driven programme, the theme is finding practical solutions to major scientific roadblocks. The questions are first identified by scientific teams, and then put to the scientific community at large. Researchers then compete for funding to pursue their unique approaches for solving the problem. Questions related to an HIV-preventive vaccine form a substantial fraction of the whole programme. An apparent extension of this approach is called the Enterprise, an alliance of groups to develop a shared strategy, and includes both the National Institute of Health and the Gates Foundation, and solely aimed at development of an HIV-preventive vaccine. Its approach will also involve competition for solving specific roadblocks to success. Another is a new and very large programme currently being developed at the National Institute of Allergy and Infectious Diseases at the National Institute of Health. The programme is called CHAVI (Center for HIV/AIDS Vaccine Initiative), and will mobilise a consortia of investigators with expertise in HIV immunology to address key issues for a successful vaccine. This approach allows these few investigators to branch out and fund other groups as needed. Whether our vaccine efforts are best approached by consortia controlled by a small elite group of researchers (the CHAVI programme); by a more open competitive process that targets very specific problems (the Gates Grand Challenges and Enterprise models); or by individual centres working closely with the National Institute of Health and large pharmaceutical companies, remains to be seen.

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Conflict of interest statement

I am a member of the Scientific Advisory Board of Neovacs, Paris, and am involved with a therapeutic Tat vaccine initiated from my own work with Daniel Zagury, Paris, which was published long before the inception of Neovacs. I am also Chairman of the Scientific Advisory Board, Profectus BioSciences, involved with the promotion of an HIV vaccine and new therapeutic HIV-preventive vaccine.

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